HIGH-THROUGHPUT PHENOTYPING BASED ON FIELD MULTISPECTRAL IMAGES ACQUISITION: Application to the evaluation of F1 hybrid apple tree response to water constraint

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1. Context and scientific questions:

There exists a variability of stomatal response under water constraint between apple or wine cultivars (Regnard et al., 2008 ; Lovisolo et al., 2010)

→ Hypothesis of isohydric *vs* anisohydric strategy in apple tree

→ Can you distinguish these strategies among F1 adult hybrids grown in field conditions?

Objectives :

- **Develop a high-throughput, relevant and sensible method for characterizing the stomatal response of a large population of apple hybrids to water constraint**
- **Reveal apple genotypic variability and perform quantitative genetic studies on this trait**

Our methodological strategy:

Phenotyping large population at field level using
- airborne images in Visible, Near-Infrared and Thermal wavebands (Vis, NIR & TIR)
- T° of transpiring surfaces referring to foliage density, represented by vegetation index (NDVI)
- estimation of leaf transpiration from TIR imaging
- Water Deficit Index (WDI) computation at tree scale

2. Water Deficit Index (WDI) concept and computation

(Moran *et al.*, 1994)

- adapted from Crop Water Stress Index: scatter plot considering T_s-T_a & vegetation cover fraction (here NDVI) as coordinates
- applicable to discontinuous cover
- varying from 0 (well-watered crop) to 1 (severely stressed)

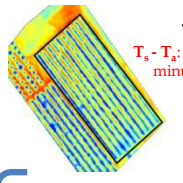
Visible image : Red, Green, Blue

NIR image

TIR image

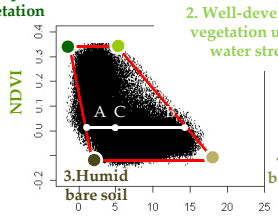
$\frac{NIR - R}{NIR + R} = NDVI$ Vegetation index

$T_s - T_a$: surface T° minus air T°



1. Well developed irrigated vegetation

2. Well-developed vegetation under water stress



- Field image pixels plotted as a function of NDVI & T_s - T_a
- Trapezoid envelope defined from quantile regression of NDVI & $T_s - T_a$
- Extreme status of transpiring surfaces corresponding to trapezoid angles

Empirical WDI equation based on the trapezoid shape (Clarke, 1997):

$$WDI = \frac{(T_s - T_a) - (T_s - T_a)_{min}}{(T_s - T_a)_{max} - (T_s - T_a)_{min}} = 1 - \frac{ET_{act}}{ET_{max}} = \frac{AC}{AB}$$

3. Field set up:

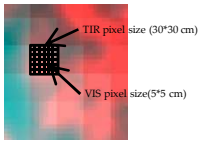
- 122 hybrids ('Starkrimson' x 'Granny Smith' progeny)
- M9 roostock
- 2 seasonal water treatments: Stressed, Non stressed (S, NS) with respect to soil Ψ
- 2 tree replicates per genotype & treatment
- → 488 apple trees on 10 rows

4. Which tree zone considering from images?

- Extraction of pure vegetation pixels depends on image resolution
- Comparison of indices values depending on the buffer size and NDVI threshold

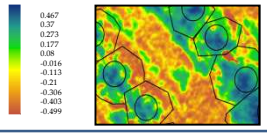
60 cm radius buffer zone at tree center

Larger buffer zone including whole tree crown + soil

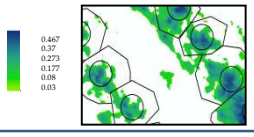


2 image treatments:

Without NDVI threshold



With threshold: NDVI > 0.03



5. Impacts of buffer size and NDVI threshold on spectral-based indices:

		With Threshold 60	With Threshold 1000		NoThreshold 60	NoThreshold 1000	
WDI	S	0.465	0.485	n.s.	0.461	0.453	n.s.
	NS	0.225	0.266	***	0.222	0.289	**
NDVI	S	0.240	0.176	***	0.228	0.082	***
	NS	0.242	0.173	***	0.239	0.111	***
T_s - T_a	S	7.048	8.057	***	7.059	9.050	***
	NS	4.380	5.627	***	4.383	6.667	***
Sdt(T_s - T_a)	S	0.775	1.851	***	0.784	2.398	***
	NS	0.786	2.187	***	0.791	2.928	***

Increasing buffer size significantly impact on NDVI and T_s - T_a, by integrating soil pixel with high T°

Accounting for a threshold reduces the impact of soil T° on indices values

→ A 60cm buffer zone with threshold was selected as the most relevant combination

6. Genotypic differences of the F1 apple population

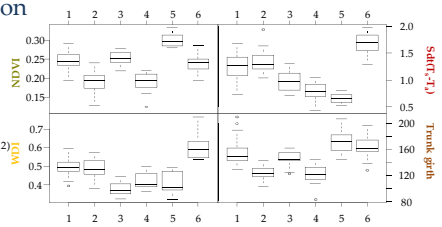
Four variables were used for characterizing the stomatal response to water constraint of apple hybrids:

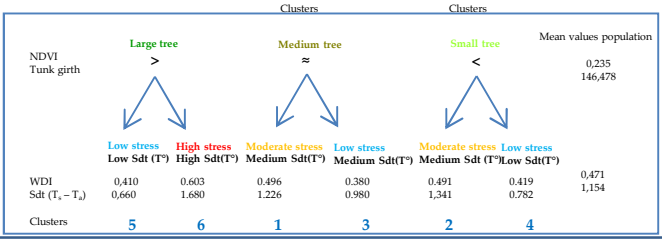
NDVI → Foliage density and nitrogen content
WDI → Water Deficit Index
Sdt(T_s-T_a) → Intra-crown foliage T°(C) variation (Gonzalez-Dugo *et al.*, 2012)
Trunk girth → Proxy of tree vigor (mm)

	NDVI	WDI	Sdt(Ts - Ta)	Trunk girth
NDVI	1	- 0.170	- 0.184	0.694
WDI	- 0.170	1	0.582	- 0.005
Sdt(Ts - Ta)	- 0.184	0.582	1	0.021
Trunk girth	0.694	- 0.005	0.021	1

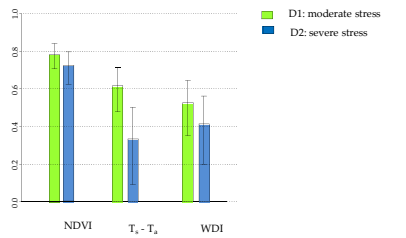
Genetic correlations (between mean genotypic values) of the variables considered for hierarchical ascendant classification

Variables are correlated 2 by2
→2 criteria for genotypes discrimination :
Tree size
Stress level





Heritability calculation for 2 successive dates with moderate (D1) and severe water constraint (D2)



- No effect of the date on NDVI ; High H^2_b
- Large effect of the date on T_s-T_a
- Higher significant genetic effect at D1 (high H^2_b), i.e. when water constraint is moderate, for both T_s -T_a and WDI

→ Moderate water constraint are more suitable for screening stomatal genotypic responses

7. Conclusions & perspectives

- Indices from remote sensing methodology are relevant for screening large genetic populations, and can be applied at individual tree scale with appropriate buffering and thresholding
- Higher heritability values were obtained with moderate water constraint
- Different clusters of genotypes were distinguished based on tree vigor and water status indices

Methodological improvements:
- Increasing TIR image resolution: UAV (Unmanned Aerial Vehicle) flight
- Comparing WDI index to variables captured *in situ* (Leaf water potential, ^{13}C)

Improve the characterization of genotypes behavior:
- Establishing a dynamic characterization during early of water stress response

Clarke, T.R. 1997. An empirical approach for detecting crop water stress using multispectral airborne sensors. HortTechnology. 7(1):9-16.
Gonzalez-Dugo, V. et al. 2012. Almond tree canopy temperature reveals intra-crown variability that is water stress-dependent. Agricultural & Forest Meteorology 154-155(1): 156-165.
Lovisolo, C. et al. 2010. Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Funct. Plant Biol. 37: 98-116
Moran, M.S., et al. 1994. Estimating crop water deficit using the relation between surface-air temperature and spectral vegetation index. Remote Sens. Env. 49:246-263.
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